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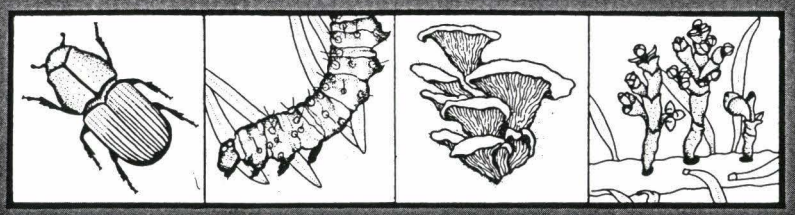

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BOTRYTIS BLIGHT OF CONTAINER-GROWN WESTERN REDCEDAR SEEDLINGS - USDA FOREST SERVICE NURSERY COEUR D'ALENE, IDAHO

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ABSTRACT

Blight caused by *Botrytis cinerea* was identified on container-grown western redcedar seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho during 1993. Stock produced in 1992 and freezer-stored for spring planting in 1993 had high levels of *B. cinerea* infection. Some lots were more damaged than others. Most infection started on primary leaves produced along the main stem of seedlings. The fungus often invaded the stem from infected primary leaves, then produced cankers that enlarged around the circumference, causing girdling. Most stem-infected seedlings had a single noticeable infection. Secondary leaf infection was common, particularly in groups of seedlings packed together for storage. *Botrytis cinerea* was also recovered from roots of more than 50 percent of seedlings with foliar or stem infections. *Botrytis* detected on stock growing under shadehouses in the fall (October - November) was concentrated on lower foliage. Producing larger seedlings in styroblock 7 (metric = 323) containers may have contributed to damage by this pathogen. Western redcedar is very susceptible to *B. cinerea* and several cultural approaches will be required to reduce future damage.

INTRODUCTION

Western redcedar (*Thuija plicata* Donn) is becoming more important in reforestation in the Northern Region. The USDA Forest Service Nursery in Coeur d'Alene, Idaho began growing this species as container stock several years ago. Demand for stock greatly increased recently, requiring expanded production. Examinations of seedlings produced during the first few crop cycles revealed they were uniform in size, of very high quality, and routinely without disease.

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During the spring of 1993, growers located foliar and stem disease on some redcedar stock produced in 1992 and freezer-stored over winter; this stock was destined for immediate outplanting when disease was detected. Since much disease was previously lacking on western redcedar seedlings at the nursery, growers were quite concerned that perhaps a new disease had been introduced. Of particular concern was the possibility of infection by Keithia blight (*Didymascella thujina* (Durand) Marie). This disease has been reported on container western redcedar in British Columbia and on bareroot redcedar in California (Dennis and Sutherland 1989; Frankel 1990; Frankel and Nelson 1991). In both situations, the disease is difficult to control and can cause extensive damage quickly.

Therefore, an evaluation was conducted on western redcedar grown and stored at the nursery to determine disease etiology, infection characteristics and disease development, and to provide guidelines for reducing future damage.

METHODS

Two groups of stock in storage were sampled for disease incidence and intensity. The first sample was 120 seedlings destined for outplanting on the Palouse Ranger District (RD)(Clearwater National Forest). This stock had been out of freezer storage for a few days prior to sampling; i.e., it was completely thawed. The other group included 30 randomly selected seedlings which were directly collected from freezer storage (not thawed); these seedlings were destined for outplanting on the Fernan RD (Idaho Panhandle National Forest) and the Lochsa RD (Clearwater National Forest).

Each sampled seedling was examined closely for disease symptoms, i.e., necrotic foliage, stem, or root lesions. For each diseased seedling, number of infections was determined by locating tissue necrosis throughout the crown. Infections originating on primary leaves (those attached directly to the stem) and secondary leaves were determined. Crowns of infected seedlings were arbitrarily divided into thirds, and the number of infections within each third was determined.

%wenty-eight seedlings with few above-ground infections were selected for root isolations of potentially pathogenic fungi. Root systems were washed thoroughly to remove growing media. Each root system was aseptically dissected into pieces approximately 3-5 mm in length. Ten pieces were randomly selected, surface sterilized in 0.525 percent aqueous sodium hypochlorite (10 percent standard bleach solution), rinsed in sterile water, and placed on selective agar medium for root pathogenic fungi (Komada 1975). Plates with roots were incubated at about 24°C for 7-10 days under diurnal cycles of cool, fluorescent light. Selected fungi emerging from root pieces were aseptically tranferred to potato dextrose agar (PDA) and carnation leaf agar (Fisher and others 1982) for identification.

Necrotic tissues from selected seedlings were assayed for presence of potentially pathogenic fungi. All sampled tissues were washed thoroughly under tap water for several minutes. Some were placed directly in moist chambers and others incubated on 2 percent water agar. Moist chambers and agar plates were incubated at about 24°C for 5-7 days and examined for fungal growth. Fungi emerging from plant tissues on agar were aseptically transferred to PDA for identification.

The 1993 crop of container western redcedar seedlings being grown under shade- houses was also examined for disease incidence. Isolations of fungi associated with foliage and stem disease were made from selected seedlings as described above.

RESULTS AND DISCUSSION

Botrytis cinerea Pers. ex. Fr. was most commonly associated with foliar and stem necrosis of western redcedar seedlings. Keithia blight was absent on sampled freezer stock and stock from shadehouses. *Botrytis* was readily isolated from nearly all necrotic tissues on redcedar seedlings. The fungus readily sporulated on infected tissues incubated in moist chambers (figure 1). More than 90 percent of the Palouse RD stock sampled, which had been removed from freezer storage for a few days prior to sampling, had evidence of foliar or stem infection by *B. cinerea* (table 1). In contrast, about 43 percent of the Fernan/Lochsa stock was infected. Seedling stem infection was very common (table 1). Most of this infection occurred via primary leaves

still attached to stems (figure 2; table 1), although some stem infection also occurred via secondary leaves. In a few cases, up to four stem infections were noted on some seedlings, although most examined seedlings had only one infection. Stem infections were located mostly in the middle third of the crown for both Palouse and Fernan/Lochsa stock. Some stem infections were found in the lower crown on the Palouse stock but not on the Fernan/Lochsa stock.

Figure 1--*Botrytis cinerea* sporulating on leaf tissue of western redcedar seedlings incubated in moist chambers. Seedlings were produced at the USDA Forest Service Nursery in Coeur d'Alene, Idaho.

Figure 2--Stem lesion caused by *Botrytis cinerea* initiated from infection of a primary leaf on western redcedar seedling at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Table 1--Stem infection of freezer-stored, container-grown western redcedar seedlings with *Botrytis cinerea* at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Percentages	
	Palouse RD[1]	Fernan/Lochsa RDs[2]
Infected Seedlings	90.0	43.3
Seedlings with Stem Infections:	35.8	26.7
One Stem Infection	22.5	23.3
Two Stem Infections	9.2	3.4
Three Stem Infections	2.5	0
Four Stem Infections	1.6	0
Source of Stem Infection:		
Primary Leaves	65.1	100.0
Secondary Leaves	34.9	0
Crown Location of Stem Infection:		
Upper Third	6.1	28.6
Middle Third	53.0	71.4
Lower Third	40.9	0

[1] Seedlings had been removed from freezer storage a few days prior to sampling; sample size = 120 seedlings.

[2] Seedlings were sampled shortly after removal from freezer storage; sample size = 30 seedlings.

Total number of *Botrytis* infections on examined stock are summarized in table 2. Most seedlings had only one infection. However, several Palouse RD seedlings had many infections; one seedling had 14 noticeable infections. In most cases, multiple infections were located on secondary leaves (figure 3), which were evident as necrotic areas sometimes with superficial mycelial growth of *Botrytis*. The Palouse RD stock had an average of one more infection per seedling than the Fernan/Lochsa stock (table 2).

Botrytis cinerea was also isolated frequently from roots of freezer-stored western redcedar seedlings (table 3). Although this fungus is normally restricted to above-ground portions of seedlings (James 1984), roots are apparently susceptible to infection. Inoculum for root infection possibly came from adjacent diseased seedlings packed for storage. If so, root infection occurred during rather than before storage. Effects of *B. cinerea* on roots of redcedar seedlings is unknown, although distinct necrotic lesions were not associated with root infection.

Botrytis cinerea was also common on 1993 stock first grown in greenhouses and later moved to shadehouses. The stock was produced in styroblock 7 (metric = 323) containers. These containers produced seedlings that were larger than those produced in the past because clients had requested extra large stock for special planting needs. Seedlings formed an extremely dense canopy on benches. Most *Botrytis* was located at the base of seedlings, sporulating on necrotic foliage; limited infection of green foliage and stem tissues was evident.

Table 2--Number of *Botrytis cinerea* infections on freezer-stored, container-grown western redcedar seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	Palouse RD[1]		Fernan/Lochsa RDs[2]	
Number of Infections	Number of Infected Seedlings	Percent	Number of Infected Seedlings	Percent
1	37	34.3	11	84.6
2	26	24.1	1	7.7
3	18	16.7	0	0
4	15	13.8	0	0
5	5	4.6	0	0
6	2	1.8	1	7.7
7	1	0.9	0	0
8	2	1.8	0	0
9	1	0.9	0	0
14	1	0.9	0	0
Totals	108	100.0	13	100.0
Avg. No. Infections Per Seedling	2.6	--	1.5	--

[1] Seedlings had been removed from freezer storage a few days prior to sampling; sample size = 120 seedlings.

[2] Seedlings were sampled shortly after removal from freezer storage; sample size = 30 seedlings.

Figure 3--*Necrotic lesion* on seconary leaf of western redcedar seedling from the USDA Forest Service Nursery, Coeur d'alene, Idaho.

Table 3--Potentially pathogenic fungi isolated from roots of freezer-stored, container-grown western redcedar seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Cylindrocarpon sp.	Percent Seedlings Infected[1]	Root Colonization Percent[2]
Botrytis cinerea	57.1	12.1
Fusarium proliferatum	7.1	1.4
Cylindrocarpon sp.	3.6	0.4

[1] Total number of seedlings sampled = 20.

[2] Percent of root pieces sampled (10 per seedling) colonized with appropriate fungus.

This evaluation indicated that container-grown western redcedar seedlings are very susceptible to *B. cinerea*. Most seedlings with advanced disease symptoms were probably culled prior to winter storage. However, culling failed to remove many infected seedlings, although most of these seedlings were only slightly infected. The fungus likely spread during some portion of the storage cycle, causing stem lesions, expanded leaf necrosis, and root infection. Although *B. cinerea* is capable of growing at cool temperatures (Lawson and Dienelt 1989; Peterson and others 1988), the fungus does not grow nor sporulate when exposed to temperatures below 0°C (McCain 1980). If storage spaces are maintained below freezing, it is expected that *Botrytis* will remain quiescent. However, temperatures within storage boxes must be maintained below freezing. Even though ambient air is below freezing, there is no guarantee that temperatures within storage boxes are the same. *Botrytis* can grow rapidly on infected seedlings in storage before temperatures are brought below freezing and once temperatures exceed freezing during thawing (McCain 1980). The humid conditions under which seedlings are stored provide an ideal environment for *Botrytis* to grow (Lopez-Herrera 1988; Smith and others 1973), although sporulation may be restricted by lack of light (Bergquist and others 1972; Honda and others 1977; Reuveni and others 1989). The fungus readily produces sclerotia (resting structures) in the dark (Hite 1973; Rotem and Aust 1991), although conidia necessary for dissemination can arise from these structures once they are exposed to light (Coley-Smith 1980).

Of major concern with *Botrytis* development on stock stored over winter is effect of the fungus on potential outplanting performance. In the past, foresters have been cautious about planting stock with noticeable disease in outplantings. In many cases, when *Botrytis* is restricted to foliage and not well developed in branches or stems, it is advisable to plant the stock (James 1984). However, when the fungus has grown into the main stem and initiated lesions, foresters may be advised to discard the seedling. Redcedar seedlings examined in this evaluation had very high levels of stem infection, although lesions usually did not extend around the stem circumference. It some cases, seedlings had multiple stem infections. The major justification for planting stock with only foliage infection has been that once the stock is placed on a forest site, the above-ground portion will sufficiently dry out so *Botrytis* will become inactive. The fungus would then be expected to die out and new foliage produced would not become infected. However, if the fungus has colonized the main stem, it is expected to continue to grow, causing an expanded lesion that may eventually girdle the stem, killing the seedling. Nevertheless, recommendations for culling *Botrytis*-infected giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchholz) have called for only discarding seedlings with stem lesions greater than 50 percent of the circumference (Smith and others 1973). Although experimental data supporting these conclusions are lacking, it is advisable to continue culling stem-infected seedlings prior to outplanting. The high costs of replanting would justify being somewhat liberal in culling stem-infecting seedlings if there is a good chance such stock would not survive.

MANAGEMENT IMPLICATIONS

The best way to prevent problems of *Botrytis* in storage is to reduce its incidence on stock before being placed into storage (James 1984; McCain 1980; Smith and others 1973). Growers at the Coeur d'Alene Nursery have extensive experience and success in controlling *Botrytis* blight on several different conifer species. The disease can be especially damaging to western larch (*Larix occidentalis* Nutt.) (Dugan and Blake 1989; James 1984) and some level of infection usually occurs despite extensive prevention efforts. Cultural techniques to reduce inoculum and provide environmental conditions less conducive to disease development are usually implemented. However, some fungicide treatments are still advised when very susceptible crops are grown (James 1984).

When *Botrytis* was first detected on redcedar seedlings, growers removed groups of heavily infected seedlings with high levels of tissue necrosis and applied spot treatment with dicloran around disease centers. This approach was largely successful in reducing fungus spread around these disease centers, but apparently did not sufficiently reduce the number of seedlings with minor infections. Because of the extreme susceptibility of western redcedar to *Botrytis*, more drastic control measures will be required to reduce damage in the future. Reduced density and height of seedlings should help control the disease by allowing foliage to become sufficiently dry between irrigations (James 1984; Mittal and others 1987). This may mean seedlings will have to be grown to smaller specifications in different types of containers so that canopies are less dense. Another alternative to improve air circulation within seedling canopies is using newly designed vented styroblock containers. These containers allow air movement from beneath benches through uniformly spaced holes adjacent to cells containing seedling root systems (Peterson and Sutherland 1990). Such containers have greatly improved air circulation and reduced length of time standing water remains on foliage following irrigation.

Fungicide treatments are probably required periodically because of the abundance of inoculum usually present in growing areas and the relative susceptibility of seedling crops (James and others 1982; Mittal and others 1987). The number of alternative fungicides available for use continues to decrease. Many chemicals are either removed from registration by companies due to public pressures or may not be re-registered for seedling use because of the high costs involved relative to limited use by the nursery industry. To compound the problem, *Botrytis* has a history of rapidly developing resistance to most chemicals once fungal populations have been exposed (Chiba and Northover 1988; Glover and others 1987; Watson and Koons 1973). Most groups of chemicals, including a wide range of benzimidazoles and carboximides, have largely become ineffective because of resistant pathogen populations (Beever and others 1991; Elad and others 1992; Leroux and others 1981). There is also evidence that *Botrytis* strains can develop resistance to multiple groups of fungicides, often with widely varying modes of action (Chastagner and Ogawa 1979; Cooley 1981; Gillman and James 1978; James and Gilligan 1983). Fungicide resistant strains may or may not exhibit decreased levels of pathogenic fitness when compared to wild type strains (Beever and others 1991; Davis and Dennis 1981). It is usually recommended that chemical fungicides be alternated to reduce selection pressures on pathogen populations to develop resistance (James 1984; McCain 1978). However, due to decreasing number of chemical options, this is becoming more difficult. Therefore, it is expected that problems of fungicide resistance will likely become more important in the future and growers will have to rely on more non-chemical methods for controlling this disease.

Fortunately, several new biological control agents show promise in controlling *Botrytis* on different greenhouse crops. Agents include specific yeasts and bacteria that show antagonism against *B. cinerea* when applied to plant foliage (McLaughlin and others 1992; Redmond and others 1987). These potential biocontrol agents are not yet available commercially, require registration, and have not been tested for efficacy on forest tree seedlings.

To reduce future damage from *Botrytis* during storage of redcedar seedlings, improved scrutiny during lift and pack operations will be required. It is important that culling eliminate most seedlings with stem infections before they are placed in storage boxes. Steps should also be taken to minimize the length of time seedlings are stored, particularly the time seedlings are held above 0°C (McCain 1980). Monitoring in-box temperatures with special probes should be routine; ambient temperatures should be adjusted to ensure that freezing

conditions prevail within boxes. After removal from storage, seedlings should be carefully examined prior to outplanting to remove those with stem infections.

Although chemical treatments have been used to successfully reduce *Botrytis* problems on certain other plants and fruit products during storage (El Ghaouth and others 1992; Hammer and others 1993), such treatments are not currently recommended for forest tree seedlings. Treatment efficacy has not been demonstrated and problems with possible exposure to chemicals by personnel handling seedlings both before and after storage should be avoided. Disease prevention is still the best alternative.

Several innovative approaches to reducing *Botrytis* problems in storage have been developed. Controlled atmospheres in storage spaces using high levels of carbon dioxide (Sitton and Patterson 1992) or sulfur dioxide (Cappellini and others 1968) have been effective in reducing pathogen activity, especially at temperatures above freezing. Fruit can be wrapped in paper impregnated with fungicidal chemicals at low concentrations which allow for extended storage and prevents *Botrytis* decay (Cappellini and others 1968). Such wrapping materials perhaps have applicability if placed inside seedling storage boxes.

CONCLUSIONS

Container-grown western redcedar seedlings are very susceptible to attack by *B. cinerea*, despite the few reports of this conifer species being seriously affected (Glover and others 1987; Peace 1962). Infection commonly occurs during seedling production and disease development may intensify during cold storage. Most grower activities during greenhouse crop production may contribute to disease enhancement by stimulating spore production and dissemination (Hausbeck and Pennypacker 1991a, b). It is important to reduce stress on seedlings during production phases; it may also be important to restrict height growth and canopy density to reduce seedling susceptibility. Altering fertilization regimes to produce less succulent seedlings may also improve disease resistance (Mittal and others 1987). A program of careful, periodic monitoring of stock during production should be an integral part of disease control programs. Sanitation is very important in preventing disease buildup (Dumroese and Wenny 1992). Removal of organic matter within greenhouses before and during crop production cycles will help reduce *Botrytis* inoculum. Surface sterilizing greenhouse interiors, including floor, walls, and benches is also important (James 1984). It may be important to periodically rotate western redcedar production into different greenhouses, especially those used to produce more *Botrytis*-resistant species such as pine and Douglas-fir. Continued production in the same greenhouses crop after crop may result in greater losses due to prevailing higher inoculum levels. Prevention and integrated control approaches should be more successful in reducing impact of *Botrytis* blight on western redcedar at the Coeur d'Alene Nursery in the future.

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